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Composition and antibacterial activity of the essential oil of six *Stachys* species from Serbia

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ABSTRACT: The essential oils of *Stachys scardica*, *S. officinalis*, *S. germanica*, *S. sylvatica*, *S. plumosa* and *S. recta* were analysed by GC–MS. From more than 100 identified constituents of the oils, the sequiterpene hydrocarbons were the major components of all samples except that of *S. plumosa*, which was rich in monoterpene hydrocarbons. Furthermore, the oils were tested against three Gram-positive and three Gram-negative bacteria. The essential oil of *S. officinalis* proved to be the most active. Copyright © 2004 John Wiley & Sons, Ltd.

KEY WORDS: *Stachys scardica*; *Stachys officinalis*; *Stachys germanica*; *Stachys sylvatica*; *Stachys plumosa*; *Stachys recta*; Labiatae; volatile constituents; Serbia

Introduction

The genus *Stachys* L. comprises more than 270 species and is considered as one of the largest genera of the family Labiatae. Serbia is an area not very rich in *Stachys* representatives. In the *Flora of Serbia*,[1] 17 species are recognized. However, eight species are endemic to the Balkan Peninsula or even narrower regions. Some species, such as *S. recta* L. and *S. alpina* L., are highly polymorphic, with a number of infraspecific taxa. Plants of this genus have long been applied in folk medicine to treat genital tumours, sclerosis of the spleen, inflammatory tumours, cough and ulcers.[2] Teas prepared from the whole plant or leaves are used in phytotherapy, possessing sedative, antispasmodic, diuretic and emmenagogue activities.[3,4] In our continuing research on the essential oils of *Stachys* spp.,[5,6] we have investigated the essential oil of *S. scardica* Griseb., belonging to the section *Macrostachya* Bhattacharjee; *S. officinalis* (L.) Trevis, belonging to the section *Betonica* (L.) Bentham; *S. germanica* L. of the section *Eriostomum* (Hoffmans & Link) Dum.; *S. sylvatica* L. of the section *Stachys*; *S. plumosa* Griseb. of the section *Swainsoniana* Bhattacharjee; and *S. recta* L. of the section *Olisia* Dum. We also report here on their antibacterial activities.

Experimental

Collection of Plant Materials

All six taxa of the genus *Stachys* were collected from natural populations as follows: *S. scardica* Griseb. and *S. plumosa* Griseb. near the city of Vranje; *S. officinalis* (L.) Trevis and *S. recta* L. on Mt Fruska Gora; *S. germanica* L. near the city of Valjevo; and *S. sylvatica* L. near the city of Beograd. To make our results comparable, only plants in similar stages of development were analysed. Voucher specimens of *Stachys* species were identified by Dr P. Marin and deposited in the Herbarium of the Institute of Botany (SGJ), Faculty of Biology, University of Belgrade, under the Accession Nos: SGJ-0020/1, SGJ-00100/1, SGJ-0130/1, SGJ-01130/1, SGJ-0150/1 and SGJ-0180/1, respectively.

Distillation and Analyses of the Essential Oils

100 g of air-dried plant material from each population were cut in small pieces, and the essential oils were obtained by steam distillation in 500 ml H_2O for 2 h,[7] in

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a modified Clevenger apparatus with a water-cooled oil receiver to reduce hydrodistillation overheating artifacts. The essential oils obtained, taken in 2 ml capillary GC grade *n*-pentane and dried over anhydrous sodium sulphate, were subsequently analysed by GC–MS and kept in closed Pyrex containers, air-tight at −4 °C. The composition of the essential oils was resolved by GC–MS analyses. Optical rotations were measured on a Perkin-Elmer 341 Polarimeter.

Gas Chromatography–Mass Spectrometry

GC–MS analyses were performed on a Hewlett-Packard 5973-6890 system operating in EI mode (70 eV), equipped with a split/splitless injector (220 °C), a split ratio 1:10, using three different columns: a fused silica HP-5 MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm); a HP-Innowax capillary column (30 m × 0.25 mm i.d., film thickness 0.50 μm) and a chiral Cydex B capillary column (50 m × 0.22 mm i.d., film thickness 0.25 μm). The temperature program for the HP-5 MS column was 60 °C (5 min) rising to 280 °C at a rate of 4 °C/min; for the HP-Innowax column, 60 °C to 260 °C at a rate of 3 °C/min; and for the Cydex B column, 50 °C to 130 °C (2 min) at a rate of 2 °C/min, then 130 °C to 250 °C at a rate of 4 °C/min. Helium was used as the carrier gas at a flow rate of 0.8 ml/min. The injection volume of each sample was 2 μl. Retention indices for all compounds were determined according to the Van den Dool approach,[8] using *n*-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of Wiley and NBS Libraries[9] and those described by Adams,[10] as well as by comparison of their retention indices with literature data.[10,11] In many cases, the essential oils were subject to co-chromatography with authentic compounds (Fluka, Sigma). The recognition of the optical isomers was made by comparison with authentic samples and according to reported elution order for the particular column.[12,13]

Biological Activity

The essential oils were tested for their antibacterial activity using a microdilution assay.[14–16] The tests were performed on 96-well micro-titre plates. The essential oils were dissolved at 10 mg/ml with DMSO and diluted with TSB (Tryptone Soya Broth, Oxoid CM 129) to a concentration of 1.0 mg/ml. The proportion of DMSO never exceeded 1% in the medium.[17] Bacterial species were cultured overnight at 37 °C in TSB. The suspensions contained ~10^9 cells/ml and adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 μl/well. Dilutions of the inocula were subcultured on TSA (Tryptone Soya Agar, Oxoid CM 131) to verify the absence of contamination and to check the validity of the inoculum. The plates were incubated for 36 h at 37 °C. DMSO was used as a control, while streptomycin was used as a positive control. The experiments for each essential oil were conducted in duplicate. Minimal inhibition concentrations (MIC) against three Gram-positive bacterial [*Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolates) and *Micrococcus flavus* (ATCC 10240)] and three Gram-negative bacteria [*Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (clinical isolates) and *Escherichia coli* (ATCC 35218)] were determined (Table 1).

Results and Discussion

All the investigated *Stachys* taxa contained essential oils in the range 0.002–0.05%, based on dry weight (Table 2). As shown in the analytical Table 2, all the essential oils are complex mixtures of more that 100 constituents in each case investigated. Among them, the sesquiterpene hydrocarbons constitute the main portion in all the taxa studied, except for *S. plumosa*, which had a high content of monoterpene hydrocarbons (73.15%). Generally, all taxa had low amounts of phenylpropanoids, fatty acids

Table 1. Antibacterial activities (MIC mg/ml) of the investigated essential oils of *Stachys* spp. and their main compounds (mg/ml)

	Staph. aureus	*B. cereus*	*M. flavus*	*E. coli*	*Pr. mirabilis*	*Ps. aeruginosa*
Streptomycin	0.1	0.1	0.1	0.2	0.4	0.5
ger	—	—	0.5	—	—	—
off	1.0	1.0	0.25	—	—	—
plu	1.0	1.0	0.5	0.25	—	—
recta	—	—	0.5	—	—	—
sca	—	—	—	—	—	—
sylv	—	—	0.5	—	—	—
β-Caryophyllene	0.1	0.1	0.1	0.2	—	—
β-Caryophyllene oxide	0.2	0.1	0.1	0.2	—	—
α-Pinene	0.2	0.1	0.1	0.2	—	—
δ-Cadinene	0.1	0.05	0.05	0.05	—	—
Linalool	0.1	0.1	0.05	0.1	—	—

germ, *S. germanica*; off, *S. officinalis*; plu, *S. plumosa*; recta, *S. recta*; sca, *S. scardica*; sylv, *S. sylvatica*.

Table 2. Chemical composition of the essential oils of *Stachys* spp.

Components[a]	RRI[b]		Section					
			Eriostomum	Betonica	Olisia	Swainsoniana	Macrostachya	Stachys
	1	2	germ*	off*	plu*	recta*	sca*	sylv*
α-Thujene	929	1032	—	—	—	—	0.12	—
(−) α-Pinene	936	1030	1.58	0.1	35.84	5.42	5.03	1.85
Camphene	954	1060	—	—	0.3	—	—	—
Benzaldeyde	959		0.64	—	—	—	—	—
Verbenene	967	1870	—	—	0.09	—	0.03	—
Octen-3-ol	977	1454	—	0.57	—	2.87	0.35	—
(+) β-Pinene	978	1122	4.28	—	31.74	8.07	0.18	1.2
Myrcene	988	1170	—	0.04	0.5	—	—	—
2-Pentylfuran	990		0.3	—	—	—	0.13	—
(*E*,*E*)-2,4-Heptadienal	1008		—	—	—	—	0.03	—
α-Terpinene	1018	1188	—	—	0.13	—	—	—
p-Cymene	1024	1280	—	—	0.08	—	0.11	—
(+) Limonene	1027	1210	0.72	—	3.62	0.44	0.09	—
(*Z*)-β-Ocimene	1038	1240	5.02	—	0.37	0.92	—	—
Phenylacetaldehyde	1041		—	—	0.05	—	0.05	—
(*E*)-β-Ocimene	1048	1260	0.69	0.15	0.11	0.91	—	—
γ-Terpinene	1059	1254	—	—	0.25	—	0.04	—
Terpinolene	1085	1290	—	—	0.12	—	0.01	—
(+) β-Linalool			—	0.32	0.12	—	—	—
(−) β-Linalool	1098	1544	0.82	—	—	—	0.15	—
n-Nonanal	1102	1387	0.49	—	0.16	0.42	0.18	—
α-Campholenal	1125		—	—	0.38	—	0.05	—
(*E*)-Pinocarveol	1134		—	—	0.31	—	—	—
(*Z*)-Verbenol	1140		—	—	0.09	—	0.05	—
(*E*)-Verbenol	1143		—	—	0.32	—	—	—
(*E*)-2-Nonenal	1160		—	—	—	—	0.04	—
Pinocarvone	1165		—	—	0.51	—	—	—
p-Mentha-1,5-dien-8-ol	1166		—	0.04	0.13	—	—	—
(+) Terpinen-4-ol	1175	1630	—	—	0.19	—	0.02	—
Myrtenal	1193		—	—	0.5	—	—	—
Myrtenol	1194		—	—	0.31	—	0.03	—
Decanal	1203		—	0.04	0.15	—	0.04	—
Octyl acetate	1214		—	0.04	—	—	—	—
β-Cyclocitral	1218		—	—	—	—	0.03	—
(*Z*)-3-Hexyl 2-methylbutanoate	1240		0.74	—	0.09	—	—	—
(*Z*)-2-Hexenyl isovalerate	1245		0.53	—	—	—	—	—
(*E*)-2-Decenal	1260		—	—	—	—	0.04	—
(*Z*)-Chrysanthenyl acetate	1267		0.89	—	—	—	—	—
Dihydroedulan II	1284		—	—	—	1.9	—	—
(−) Bornyl acetate	1285	1590	—	—	0.41	—	—	—
Dihydroedulan I	1289		—	0.04	—	—	0.19	—
Carvacrol	1297	2215	1.59	—	0.05	—	0.03	—
Tridecane	1300		—	—	0.06	—	0.06	—
(*E*,*E*)-2,4-Decadienal	1315		—	—	0.07	—	0.06	—
Octyl isobutyrate	1336		1.18	—	—	—	—	—
α-Cubebene	1348	1468	—	0.76	0.1	—	1.56	0.17
Eugenol	1353		0.36	0.07	0.1	—	0.03	—
(*E*)-2-Undecenal	1364		—	—	—	—	0.03	0.35
α-Ylangene	1372		0.72	0.42	—	—	1.26	—
(−) α-Copaene	1375	1535	—	0.25	0.64	—	2.79	0.4
β-Bourbonene	1387	1552	0.86	1.88	0.08	2.15	2.62	0.4
Benzyl isovalerate	1385		0.94	—	—	—	—	—
β-Cubebene	1388	1548	—	0.42	—	—	0.61	—
(−) β-Elemene	1391	1598	0.45	0.45	—	1.9	—	0.33
Dodecanal	1407		—	—	—	—	—	0.46
α-Cedrene	1408		—	—	—	—	0.09	—
(*Z*)-α-Bergamotene	1413		0.23	—	—	—	0.28	—
(−) (*E*)-Caryophyllene			—	—	0.73	—	—	—
(+)(*E*)-Caryophyllene	1418	1607	0.32	—	—	20.37	2.23	18.14
Octyl 2-methyl butyrate	1421		2.8	—	—	—	—	—
Calarene	1432		—	—	—	—	2.42	0.06
(*E*)-α-Bergamotene	1434	1590	—	1.19	0.21	—	—	—
γ-Elemene	1437		—	—	—	—	0.22	—
Aromadendrene	1439		—	0.08	—	—	0.32	—
(*Z*)-β-Farnesene	1445		—	0.15	0.12	—	0.08	—
α-Humulene	1452	1678	—	4.4	—	1.91	0.18	2.5
Geranyl acetone	1453		0.26	—	0.12	—	0.24	—

Table 2. (*Continued*)

Components[a]	RRI[b]		Section					
			Eriostomum	Betonica	Olisia	Swainsoniana	Macrostachya	Stachys
(*E*)-β-Farnesene	1457		13.26	—	0.57	0.42	0.2	—
allo-Aromadendrene	1458		—	—	—	—	—	0.55
γ-Gurjunene	1473		0.24	—	—	—	—	—
γ-Muurolene	1477	1704	—	—	—	—	1.56	—
Germacrene D	1480	1726	16.49	42.7	0.24	27.06	0.15	19.08
ar-Curcumene	1481	1762	—	—	—	—	0.45	—
α-Amorphene	1485	1678	—	3.93	0.12	—	10.01	4.91
β-Selinene	1487	1751	—	—	—	—	0.43	2.22
(*E*)-β-Ionone	1489		—	—	0.2	1.09	—	1.57
Phenyl ethyl 3-methyl butanoate	1489		2.91	—	—	—	—	—
epi-Bicyclosesquiphellandrene	1490		—	0.28	—	—	0.50	0.37
Valencene	1491		0.21	—	—	—	—	—
Bicyclogermacrene	1494		2.4	—	—	2.71	—	—
α-Zingiberene	1495	1713	—	—	0.41	—	—	—
γ-Amorphene	1496		—	—	—	—	3.35	—
α-Muurolene	1499	1735	0.29	1.28	—	—	2.37	1.05
Gerrnacrene A	1506		—	0.47	—	10.38	—	—
(*E*,*E*)-α-Farnesene	1508	1742	0.41	—	0.29	—	0.11	2.03
γ-Cadinene	1513	1770	0.21	5.01	—	—	6.2	—
7-*epi*-α-Selinene	1516	1762	—	—	—	1.02	—	—
δ-Cadinene	1524	1767	1.72	6.3	0.58	0.76	10.13	6.4
Cadina-1,4-diene	1531	1768	—	0.41	0.14	—	0.41	0.23
(*E*)-γ-bisabolene	1535		—	—	—	—	2.34	—
α-Cadinene	1537		—	0.47	—	—	0.75	1.42
α-Calacorene	1540	1916	—	0.19	0.09	—	1.48	0.17
β-Calacorene	1561		—	0.09	—	—	0.7	10.4
(*E*)-Nerolidol	1564	1898	1.3	0.08	1.12	1.23	—	—
α-Cedrene epoxide	1570		—	0.12	0.1	—	—	—
Germacrene-D-4-ol	1574		0.38	0.70	—	—	—	0.18
Spathulenol	1577	2135	0.81	—	0.39	—	0.8	—
(+) Caryophyllene oxide	1581	1990	—	1.68	1.03	2.83	1.9	6.18
Salvial-4(14)-en-1-one	1589		—	0.22	—	—	2.63	0.41
Carotol	1594		—	0.43	—	—	—	—
2-Acetyl-naphthalene	1604		7.21	—	—	—	—	—
β-Oplopenone	1608		—	—	—	—	0.55	—
Humulene epoxide	1606		—	—	—	—	—	0.36
1-*epi*-Cubenol	1627		—	—	—	—	2.14	0.33
epi-α-Cadinol	1637	2185	0.81	0.28	0.68	—	1.99	1.52
epi-α-Muurolol	1640		1.47	1.25	—	—	1.28	—
Torreyol	1646		—	0.39	0.29	—	4.07	1.17
α-Cadinol	1656		—	2.33	—	—	5.16	1.3
Valeranone	1672		—	0.25	—	—	—	—
β-Bisabolol	1673		—	—	—	—	1.73	—
α-Bisabolol	1682	2012	—	—	1.16	—	—	—
Phenyl ethyl heptanoate	1740		13.75	—	—	—	—	—
Benzyl benzoate	1762		—	—	0.07	—	—	—
Myristic acid	1798		—	0.08	0.12	—	0.11	—
6,10,14-Trimethyl pentadecan-2-one	1845		0.39	0.12	0.41	—	0.18	—
Farnesyl acetone	1914		—	0.05	0.13	—	0.07	—
ent-Pimara-8,15-diene	1942		—	—	0.14	—	—	—
13-*epi*-Manool	1961		1.47	—	0.08	—	—	—
Hexadecanoic acid	1972		—	0.62	—	—	—	—
Manoyl oxide	1994		—	—	0.47	—	—	—
Kaurene	2043		—	—	0.09	—	—	—
Abietatriene	2058		—	—	3.47	—	—	1.69
Heneicosane	2100		0.39	—	—	—	—	—
(*E*)-Phytol	2135		—	0.18	0.24	—	0.06	1.1
Sclareol	2229		—	—	—	—	—	1.03
Tricosane	2300		—	—	—	0.22	—	—
Abieta-7,13-dien-3-one	2315		—	—	0.74	—	—	—
Tetracosane	2400		0.03	—	—	—	—	—
Pentacosane	2500		—	—	—	0.09	—	—
Total			92.14	81.32	92.83	94.78	86.33	91.53
Yield (% v/dry weight)			0.02	0.04	0.05	0.02	0.02	0.002
$[\alpha]_D^{20}$			−12.0 ($CHCl_3$, ca. 0.27)	−72.9 ($CHCl_3$, ca. 0.98)	+2.5 ($CHCl_3$, ca. 0.93)	−0.8 ($CHCl_3$, ca. 0.76)	−1.5 ($CHCl_3$, ca. 0.48)	−21.6 ($CHCl_3$, ca. 0.04)

Table 2. (*Continued*)

Components[a]	RRI[b]	Section					
		Eriostomum	Betonica	Olisia	Swainsoniana	Macrostachya	Stachys
Grouped components		germ	off	plu	recta	sca	sylv
Aliphatics							
Alkanes, alkenes		—	—	0.37	—	0.48	—
Alcohols		—	0.57	—	2.87	0.35	—
Aldehydes		1.13	0.04	0.38	0.42	0.42	0.81
Ketones		0.65	0.17	0.66	—	0.49	—
Fatty acids		—	0.7	0.12	—	0.11	—
Esters		23.74	0.04	0.57	—	—	—
Terpenoids							
Monoterpene hydrocarbons		12.29	0.29	73.15	15.76	5.61	3.05
Oxygenated monoterpenes		0.82	0.36	2.86	—	0.33	—
Sesquiterpene hydrocarbons		37.81	71.13	4.32	68.68	55.8	70.83
Oxygenated sesquiterpenes		4.77	7.73	4.77	4.06	22.25	11.45
Diterpenoids		1.47	0.18	5.23	—	0.06	3.82
Phenylpropanoids		1.95	0.07	0.2	—	0.11	—
Other compounds		7.51	0.3	—	—	0.13	—

[a] Components listed in order of elution from a HP 5MS column.
[b] RRI, relative retention indices calculated against C_9–C_{24} *n*-alkanes on the HP 5MS (1). and HP Innowax (2) capillary columns, respectively.
* For species abbreviations, see Table 1.

and aliphatic esters, with the exception of *S. officinalis*, which was rich in esters (23.74%). The use of a chiral column allowed the determination of enantiomers in several main compounds. In most cases only one isomer was present in the essential oil, the other being absent or present in trace amount.

In spite of the large size of the genus *Stachys*, the composition of volatile compounds is known in only a small number of species. The previously studied essential oil of *S. recta* from Serbia[18] resembles that from Turkey[19] in the preponderance of 1-octen-3-ol, while in the present essential oil of *S. recta* germacrene D and (+) (*E*)-caryophyllene were the main compounds. Isocaryophyllene and β-caryophyllene, dominating constituents of the previously investigated essential oil of *S. officinalis* from Montenegro,[20] were totally absent in the present sample, with germacrene D being the main compound. Stachynone and stachynene, main constituents of the essential oils of *S. sylvatica* and *S. recta*,[21] were absent in the essential oils studied.

The essential oils investigated showed better activity against Gram-positive than against Gram-negative bacteria. The essential oil of *S. officinalis* proved to be the most active. *Pseudomonas aeruginosa* and *Proteus mirabilis* were found to be the most resistant strains, as neither of the essential oils was active against them. Knobloch *et al*.[22] showed that oxygenated monoterpenes, such as linalool, exhibit strong antimicrobial activity, especially pronounced on whole cells, while hydrocarbon derivatives possess lower antimicrobial properties, as their low water solubility limits their diffusion through the medium. Griffin *et al*.[23] have shown that hydrocarbons tend to be relatively inactive regardless of their structural type, and this inactivity is closely related to their limited hydrogen bound capacity and water solubility. Ketones, aldehydes and alcohols are active, but with differing specificity and levels of activity, which is related to the present functional group, but also associated with hydrogen-bounding parameters in all cases. Previous results showed that greater antimicrobial potential could be ascribed to the oxygenated terpenes.[22,24,25]

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